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Pharmacodynamics and pharmacokinetics of carprofen, a non-steroidal anti-inflammatory drug, in healthy cows and cows with *Escherichia coli* endotoxin-induced mastitis

J. A. C. M. LOHUIS, T. VAN WERVEN, A. BRAND, A. S. J. P. A. M. VAN MIERT,* E. ROHDE,† B. LUDWIG,† P. HEIZMANN† & W. F. REHM†

Department of Herd Health and Reproduction, and *Department of Basic Sciences, Division of Pharmacology, Pharmacy and Toxicology, College of Veterinary Medicine, State University of Utrecht, Yalelaan 7, NL–3584 CL Utrecht, The Netherlands, and †F. Hoffmann–La Roche Ltd, Pharmaceutical Research Department, CH–4002 Basel, Switzerland

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The pharmacodynamics of carprofen and its pharmacokinetics in plasma and milk of healthy cows and cows with endotoxin-induced mastitis were studied after a single intravenous dose of 0.7 mg/kg body weight. Carprofen was administered to five clinically healthy cows and to the same cows 3 weeks later, 2 h after intramammary infusion of endotoxin. Mastitis developed in all endotoxin-infused quarters. The pharmacokinetic characteristics of carprofen in healthy cows were a small volume of distribution (0.09 l/kg), a relatively low systemic clearance (2.4 ml/h kg), and a long elimination half-life (30.7 h). In the mastitic cows, systemic clearance (1.4 ml/h kg) was significantly lower ($P < 0.01$), and elimination half-life (43.0 h) was significantly longer ($P < 0.01$) than in the normal animals. Concentrations of carprofen in milk from healthy quarters were below the limit of detection for the assay (0.022 μg/ml). In milk from mastitic quarters, concentrations of carprofen increased up to 0.164 μg/ml during the first 12 h after induction of mastitis, but were less than 0.022 μg/ml at 24 to 48 h. Compared with the untreated mastitic controls, carprofen treatment significantly reduced heart rate ($P < 0.01$), rectal temperature ($P < 0.001$), quarter swelling ($P < 0.01$) and other parameters measured. Local and systemic adverse reactions to carprofen were not observed.

J. A. C. M. Lohuis, Department of Herd Health and Reproduction, Division of Pharmacology, Pharmacy and Toxicology, College of Veterinary Medicine, State University of Utrecht, Yalelaan 7, NL–3584 CL Utrecht, The Netherlands.

INTRODUCTION

Carprofen ((±)-6-chloro-α-methylcarbazole-2-acetic acid) is a novel non-steroidal anti-inflammatory drug (NSAID) that is well-tolerated in various species (Strub *et al.*, 1982; Teelmann, 1983; Ludwig *et al.*, 1989). In rats and mice the drug has a similar

analgesic, antipyretic, and anti-inflammatory activity to indomethacin. However, in these species the drug is more potent than phenylbutazone and is better tolerated than many other currently used NSAIDs (Strub *et al.*, 1982; Randall & Baruth, 1976). The S enantiomer of carprofen, which is available as a racemic mixture, was shown to be approximately 16 times more potent than the R enantiomer by using *in vitro* and *in vivo* tests (Gaut *et al.*, 1975). This is not a large difference of activity in comparison to what was already observed in some other aryl propionic acid NSAIDs (Hutt & Caldwell, 1984). Therefore, carprofen may be used as a racemate since no great advantages in form of activity would be obtained by using the pure S enantiomer.

NSAIDs have been used in therapy for acute or peracute bovine mastitis (Eberhart *et al.*, 1979; Lohuis *et al.*, 1989). In a pilot experiment using two cows, carprofen exhibited an elimination half-life ($t_{1/2\beta}$) of 28.5 h and 41.5 h after a single intravenous injection of 0.7 mg/kg body weight. Further studies demonstrated that the concentration of carprofen in milk was below the quantification limit of the analytic method (0.022 μg/ml) during the treatment and up to 5 days after the last injection of carprofen; in contrast, mastitic milk showed concentrations of carprofen up to 0.3 μg/ml (Ludwig *et al.*, 1989).

The aims of this study were to determine the pharmacokinetics of carprofen in plasma and milk of healthy cows and cows with endotoxin-induced mastitis after a single intravenous dose and to establish its pharmacodynamic effects in mastitic cows. The model was intended to approximate clinical conditions that were severe enough to evaluate the influence of drug treatment. Therefore, carprofen was injected at 2 h after intramammary infusion of endotoxin, that is, when quarter swelling was obvious and rectal temperatures were at least 1°C above baseline values.

MATERIALS AND METHODS

Animals

Eleven clinically healthy, adult, Holstein × Dutch Friesian (n = 9) or Meuse Rhine Yssel (n = 2) cows were used. Cows were in their first to sixth lactation and 28 to 156 days after calving. They were kept in stalls and fed wilted grass silage, or fresh grass, and concentrate according to their milk production. Water was provided *ad libitum*. Seven days and 4 days, and 24 h and 2 h before the start of each trial the cows' udders were examined clinically, and quarter foremilk samples were taken for diagnostic bacteriological examination according to the instructions given by the National Mastitis Council for the USA (1981) and somatic cell counts according to the recommended method for counting by the International Dairy Federation (1986) (Model F; Coulter Electronics, UK).

Induction of Escherichia coli *endotoxin mastitis*

Purified lipopolysaccharide (LPS) obtained from *E. coli* 0111:B4 (lot 667697, Difco Laboratories, Detroit, MI, USA) was used to induce experimental mastitis as described by Verheijden *et al.* (1982). Immediately prior to infusion, 0.1 mg of LPS was dissolved in 20 ml of pyrogen-free saline. The endotoxin was infused in rear quarters using an 18 gauge milking cannula 1 h after the morning milking.

Test preparation

A 5% (w/v) carprofen formulation (referenced as Ro 20-5720/656, batch No G PH 14 161, F. Hoffmann–La Roche Ltd, Basel, Switzerland) was injected as a bolus over a period of 10 s into the right jugular vein at a dose rate of 0.7 mg/kg body weight.

Assessment of clinical parameters

Clinical parameters were determined every 30 min from 3 h before infusion of endotoxin or injection of carprofen (baseline values) up to 12 h after injection of carprofen; they were also determined at 15 h, or 16 h, 24 h and 48 h after injection of carprofen.

Rectal temperature and heart rate were determined as previously described (Lohuis *et*

al., 1989). General attitude and quarter swelling of rear quarters were subjectively assessed using the scoring system according to Anderson *et al.* (1986). Scores for general attitude were: 1 = normal attitude; 2 = moderate depression (decreased appetite, reduced activity and rumination); and 3 = severe depression (no appetite, very dull appearance, absence of rumination and sometimes salivation). The quarter swelling was evaluated by palpation by hand. The following scores were used: 1 = no swelling; 2 = moderate swelling or tenderness; and 3 = marked swelling and very sore to touch, leakage of milk in most cases. Scores for quarter swelling of left and right rear quarters were given as the mean of at least two measurements for each cow.

Experimental procedures

In the first study, five healthy cows were used to investigate the pharmacokinetics of carprofen following intravenous administration of 0.7 mg/kg body weight. Carprofen was injected 2 h after the morning milking.

In the second trial involving the same animals, mastitis was induced by intramammary infusion of endotoxin into both rear quarters of each cow. Carprofen was injected approximately 2 h after infusion of endotoxin. At that time, swelling of the infused quarters was obvious and rectal temperatures were at least 1°C above pre-infusion baseline values. The interval between trials one and two was 3 weeks. For quality control of the mastitis model, mastitis was induced in six cows without carprofen treatment.

Duplicate blood samples (10 ml) for carprofen assay in plasma were collected from the left jugular vein using vacutainer tubes containing potassium-ammonium-oxalate as anticoagulant. Samples were taken before drug administration and at various times up to 120 h after injection of carprofen. Blood was immediately centrifuged upon collection (15 min, 1700 ***g***) and the plasma was separated and deep-frozen at −20°C until analysis.

Milk for carprofen assay was collected (10 ml) in sterile polyethylene tubes (Sterilon®, Continental Pharma Zutphen, The Netherlands). Foremilk was sampled from each quarter before infusion of endotoxin, before injection of carprofen, at 2, 6, 12, 15 and 18 h after carprofen injection, and thereafter at milking times (21, 29, 45, 72, 96, and 118 h after injection of carprofen). Part of each foremilk sample was used for cell counting and part for electronic measurement of the pH value.

To elucidate the distribution of carprofen within the milk fractions from the quarters, extra samples were collected at milking times. After cows were milked, a sample from the milk of each quarter was collected from the contents of the milking machine after mixing (mixed milk). Moreover, milk was taken from each quarter after cows were milked (aftermilk).

Assays

Plasma and milk were assayed for carprofen in duplicate by means of a high-performance liquid chromatography (HPLC) method with fluorescence detection using a normal-phase technique as described in detail by Ascalone & Dal Bo (1983). In this investigation the limit of quantification of carprofen was 0.040 µg/ml in plasma and 0.022 µg/ml in milk, with an accuracy and precision better than 10%. Plasma and milk samples spiked with known quantities of carprofen were analysed along with the unknown samples in order to check the validity and precision of the method during the routine analysis.

Pharmacokinetic and statistical analyses

Pharmacokinetic analyses of the data were performed with the aid of a computer program for extended least-squares non-linear regression analysis (ELSFIT) (Peck *et al.*, 1984; Sheiner & Beal, 1985).

The data were analysed using a two-compartment open model for both healthy and mastitic cows, with the exception of one healthy cow for which a three-compartment model was fitted to the plasma concentration/time data. The plasma concentrations were described by the bi-exponential equation (Gibaldi & Perrier, 1982):

$$C_t = Ae^{-\alpha t} + Be^{-\beta t}$$

where C_t = plasma concentration at time t
A, B = intercept terms
e = base of the natural logarithm
α = distribution rate constant
β = elimination rate constant

Values for A, B, α, β, k_{12} (rate constant for transfer from compartment 1 to 2), k_{10} (rate constant for transfer from compartment 1 out of the system), k_{21} (rate constant for transfer from compartment 2 to 1), the distribution half-life ($t_{1/2}\alpha$), the elimination half-life ($t_{1/2}\beta$), the volume of the central compartment (V_c), the volume of distribution (area) (V_d), the volume of distribution at steady state (V_{ss}) ($V_{ss} = V_c \times (1 + k_{12}/k_{21})$), the body clearance ($Cl_B$) and the area under the curve ($AUC_{0-\infty}$) ($AUC = A/\alpha + B/\beta$) were determined (Table I) (Gibaldi & Perrier, 1982).

The mean pharmacokinetic parameters were obtained from the average of the individual plasma concentrations of the cows. Significance of difference was tested with Student's paired *t*-test, or independent *t*-test. The 'null' hypothesis was rejected at the 5% or 1% level. Although all parameters were evaluated statistically, it was recognized that this might only be appropriate for the directly fitted parameters A, B, α, and β.

RESULTS

The clinical examination of the cows before the trials revealed no abnormalities. All quarters were free from mastitis pathogens, and foremilk somatic cell counts (SCC) were less than 500 000/ml. Average pH values of foremilk samples were 6.59 (range 6.48 to 6.68) for rear quarters, and 6.51 (range 6.36 to 6.66) for front quarters.

The mean rectal temperature of all cows at the start of the trials was 38.7°C (range 38.4°C to 39.5°C, four observations per cow); the mean heart rate was 84 beats/min (range 72 to 92). The average milk production was 25.3 l/day (range 19.1 to 32.0 l/day, seven observations per cow); the milk production in rear quarters was 56.1% of total daily milk yield (range 50.0 to 61.8%).

The response of the untreated cows to intramammary infusion of endotoxin (Figs 1–5) included fever (maximum temperature was

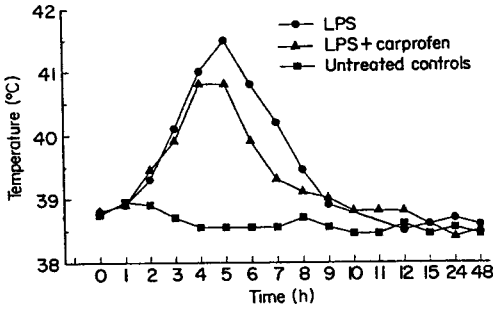

FIG. 1. Mean rectal temperatures in healthy (n = 5) and mastitic cows (n = 5) receiving carprofen, and in untreated mastitic cows (n = 6). Mastitis was induced by intramammary infusion of 0.1 mg lipopolysaccharide of *Escherichia coli* (LPS) into rear quarters. Carprofen (0.7 mg/kg body weight) was injected intravenously at post-infusion hour (PIH) 2.0 in the mastitic cows.

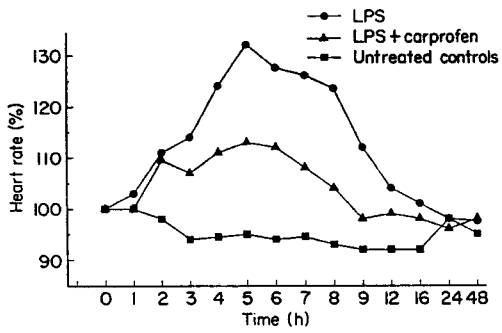

FIG. 2. Mean heart rates (as percentage of baseline values) in healthy (n = 5) and mastitic cows (n = 5) receiving carprofen, and in untreated mastitic cows (n = 6). Mastitis was induced by intramammary infusion of 0.1 mg lipopolysaccharide of *E. coli* (LPS) into rear quarters. Carprofen (0.7 mg/kg body weight) was injected intravenously at post-infusion hour (PIH) 2.0 in the mastitic cows.

41.5 ± 0.5°C), tachycardia (maximum values were 127 ± 8% of baseline), general depression (decreased appetite, dull appearance, decreased activity) and a marked inflammatory response of the infused quarters (heat, swelling, pain). Rectal temperature and heart rate returned to the baseline values at post-infusion hours 10 and 12, respectively.

The pH values in foremilk samples from rear quarters of healthy and mastitic cows receiving carprofen in relation to plasma concentration and pH values in foremilk

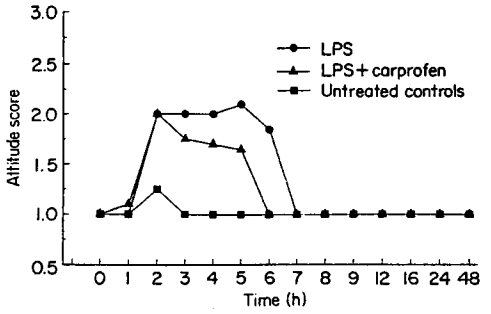

FIG. 3. Mean scores for general attitude in healthy (n = 5) and mastitic cows (n = 5) receiving carprofen, and in untreated mastitic cows (n = 6). Mastitis was induced by intramammary infusion of 0.1 mg lipopolysaccharide of *E. coli* (LPS) into rear quarters. Carprofen (0.7 mg/kg body weight) was injected intravenously at post-infusion hour (PIH) 2.0 in the mastitic cows.

samples of untreated mastitic cows are shown in Fig. 5.

In healthy cows receiving carprofen the pH values were slightly decreased. The corresponding values in mastitic cows increased 3 h after induction of mastitis.

Systemic signs in mastitic cows treated with carprofen showed a similar pattern (Figs 1–4). However, maximum values for rectal temperature (40.8 ± 0.54°C) and for heart rate (111 ± 12% of baseline) were significantly lower ($P < 0.001$ and $P < 0.01$ respectively) in mastitic cows receiving carprofen treatment. Compared with untreated mastitic cows, carprofen treatment significantly ($P < 0.01$) reduced the general depression between 5 h and 9 h after induction of mastitis and the swelling of the mastitic quarters from 5 h to 13 h after injection of carprofen.

Mean plasma concentrations of carprofen in healthy and mastitic cows are given in Fig. 6. The pharmacokinetic parameters of carprofen in healthy and mastitic cows are shown in Table I. The main plasma disposition characteristics of carprofen in healthy cows after intravenous injection were a small volume of distribution (V_d = 0.091 ± 0.003 l/kg body weight), a rather low systemic clearance (Cl_s = 2.4 ± 0.16 ml/h kg), and a long plasma elimination half-life of 30.7 h (range 23.6 to 37.7). In mastitic cows the volume of distribution was 0.086 ± 0.004 l/kg body weight (not significantly different from healthy cows, $P > 0.05$). However, Cl_s was significantly lower at 1.4 ± 0.13 ml/h kg ($P < 0.01$), and plasma elimination half-life was significantly longer, 43.0 h (range 39.2–51.8; $P < 0.01$), in the mastitic cows.

The concentration of carprofen in milk from healthy cows was below the detection limit of 0.022 μg/ml. This clearly indicates that carprofen was not excreted into the milk of healthy cows to any great extent. However, in the foremilk samples from mastitic quarters 12 h after injection the concentration of carprofen increased up to 0.164 μg/ml (Table II), whereas concentrations in non-inflamed front quarters remained below the quantification limit. This indicates a rapid diffusion of carprofen across the blood/milk barrier in mastitic quarters.

Only small differences were obtained between concentrations of carprofen in foremilk, mixed milk and aftermilk collected from inflamed quarters (Table II). The concentration of carprofen increased 2 h after intravenous injection, and was below or near to the quantification limit from 48 h after drug administration. A low concentration (0.031 μg/ml) of carprofen was quantified only in one cow in aftermilk at 72 h after injection.

DISCUSSION

In laboratory animals and in horses, inhibition of prostaglandin synthesis by carprofen was slight in relation to its anti-inflammatory and analgesic potency in experimental models (Strub *et al.*, 1982; P. Lees, pers. comm.). This indicates that another mechanism of action must exist to explain the anti-inflammatory effect of carprofen e.g. lipoxygenase inhibition. It is, therefore, difficult to establish a correct dose based on inhibition of prostaglandin synthesis only.

In the present experiment carprofen induced a significant reduction in rectal temperature, heart rate and quarter swelling in cows with endotoxin-induced mastitis (Figs 1, 2 & 4) from 1 h to 9 h after intravenous injection. During the observed effective period (at least 9 h), plasma concentrations of carprofen ranged between 11.6 μg/ml and 6.5 μg/ml (Fig. 6). Studies in laboratory animal species indicate that considerably higher doses of

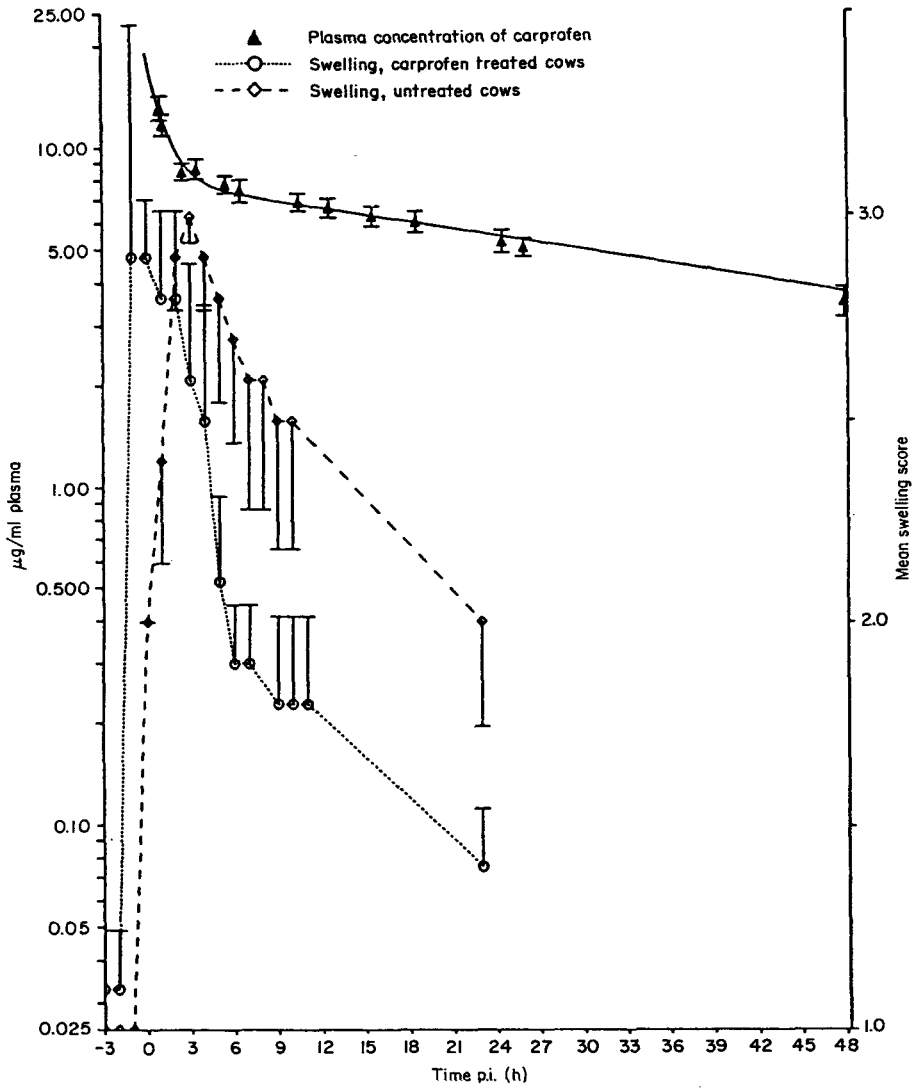

FIG. 4. Mean plasma concentration (± SEM) of carprofen in mastitic cows, and mean swelling scores (± 95% confidence intervals) for rear quarters of mastitic cows receiving carprofen and untreated mastitic cows.

NSAIDs are required to prevent oedema and leukocyte infiltration compared with those required to block the generation of eicosanoids (Lees *et al.*, 1988). This indicates that clinical evaluation remains necessary to demonstrate the value of NSAIDs for their therapeutic efficacy in a specific disease.

Literature on the pharmacokinetics of NSAIDs in diseased animals is sparse. Therefore, we studied the pharmacokinetics of carprofen in cows with *E. coli* endotoxin-induced mastitis. The pharmacokinetic parameters obtained for healthy and mastitic cows showed some differences: in the mastitic cows, the $t_{1/2\alpha}$ and the Cl_s of carprofen were significantly lower, and the elimination half-life ($t_{1/2\beta}$) was significantly longer (Table I). Since many factors can affect plasma levels of drugs in disease states, one must be very cautious in explaining changes in distribution

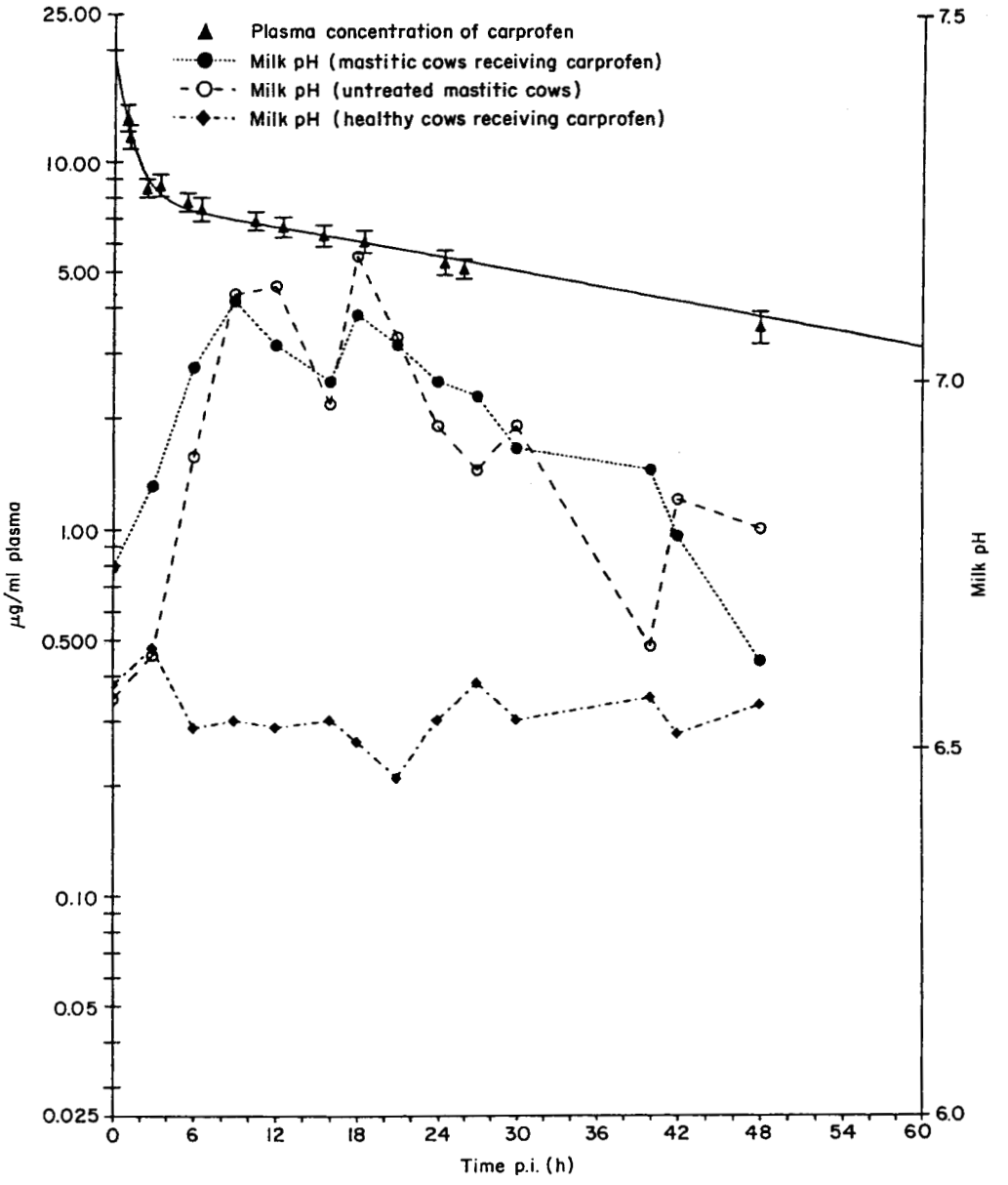

FIG. 5. Mean plasma concentration (± SEM) of carprofen in mastitic cows, and foremilk pH in healthy cows (n = 5), in mastitic cows receiving carprofen (n = 5) and untreated mastitic cows (n = 6).

and elimination. The increased $t_{1/2\beta}$ observed in the mastitic cows may be explained by peripheral vasoconstriction and redistribution of the circulating blood to the central compartment that occur during the onset of febrile states (Blatteis *et al.*, 1988). As for most weakly acidic drugs, the volume of distribution of carprofen is small, indicating a low degree of tissue uptake of the drug. The lower systemic clearance of drugs during febrile conditions may be caused by changes in hepatic and renal blood flow (Blatteis *et al.*,

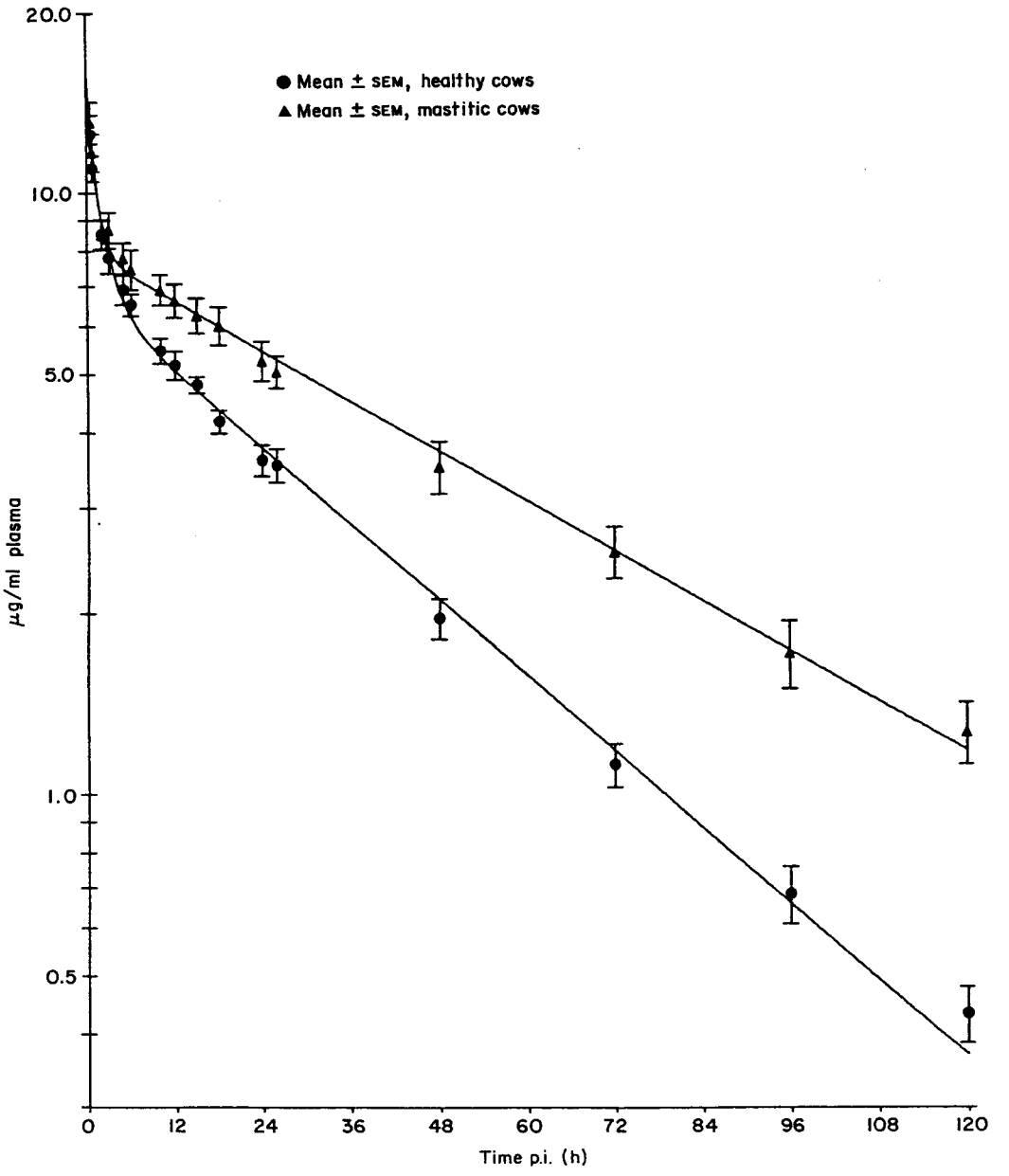

FIG. 6. Carprofen in healthy cows and in cows with endotoxin-induced mastitis: mean plasma concentration following the intravenous injection of 0.7 mg/kg.

1988) and/or impaired hepatic drug metabolism (Abdullah & Baggott, 1986; Van Gogh *et al.*, 1989).

Concentrations of carprofen in the milk from inflamed mastitic quarters were increased (up to 0.164 ± 0.024 µg/ml) but were still lower than plasma concentrations indicating that carprofen was not concentrated within the udder. Comparison of the data in Fig. 5 and Table II shows that the period with increased concentration of carprofen in milk coincides with the period during which pH

TABLE I. Comparison of mean pharmacokinetic parameters (± SEM) of carprofen in healthy (n = 4) and mastitic cows (n = 5) following intravenous administration of 0.7 mg/kg body weight

Pharmacokinetic parameter		Healthy cows		Mastitic cows		P
V_c	(l/kg)	0.051	(0.004)	0.047	(0.002)	NS
A	(mg/l)	7.09	(0.91)	6.94	(0.32)	NS
α	(l/h)	0.43	(0.087)	0.82	(0.21)	NS
$t_{1/2\alpha}$	(h)	1.81	(0.36)	1.01	(0.16)	*
B	(mg/l)	6.84	(0.15)	7.91	(0.39)	*
β	(l/h)	0.023	(0.002)	0.016	(0.0008)	**
$t_{1/2\beta}$	(h)	30.7	(2.3)	43.0	(2.3)	**
k_{21}	(l/h)	0.224	(0.040)	0.432	(0.095)	NS
k_{10}	(l/h)	0.047	(0.004)	0.030	(0.002)	**
k_{12}	(l/h)	0.188	(0.047)	0.374	(0.118)	NS
Vd_{ss}	(l/kg)	0.091	(0.003)	0.086	(0.004)	NS
AUC	(mg h/l)	294.3	(19.2)	507.4	(52.1)	**
Cl_s	(ml/h kg)	2.4	(0.16)	1.4	(0.13)	**

t-test; * = $P < 0.05$; ** = $P < 0.01$; NS = not significant. Microconstants were calculated by compartmental analysis of the plasma concentration–time data. A two-compartment model was used for pharmacokinetic analysis.

TABLE II. Mean carprofen concentrations (± SEM) in foremilk, mixed milk and aftermilk from inflamed quarters of five cows with endotoxin-induced mastitis following intravenous injection of 0.7 mg carprofen/kg body weight

Time p.i. (h)	Foremilk (µg/ml)	n	Mixed milk (µg/ml)	n	Aftermilk (µg/ml)	n
0	< 0.022	10	n.s.		n.s.	
2	0.147 (0.034)	8	n.s.		n.s.	
6	0.151 (0.026)	10	n.s.		n.s.	
12	0.164 (0.024)	10	n.s.		n.s.	
15	0.147 (0.019)	10	n.s.		n.s.	
18	0.130 (0.023)	10	n.s.		n.s.	
21	0.096 (0.020)	10	0.106 (0.022)	10	0.105 (0.018)	10
29	0.054 (0.010)	10	0.060 (0.011)	8	0.066 (0.014)	10
45	0.027 (0.001)	6	0.050 (0.012)	3	0.058 (0.010)	5
72	< 0.022	10	< 0.022	10	< 0.022	10

n = number of quarters; n.s. = no sample collected.

values of mastitic milk were highest. However, the pKa of carprofen is 4.7. Therefore, it may be expected that the driving force for the excretion of carprofen in the milk is the increase of the pH in the milk. Our results suggest that carprofen concentrations in mastitic milk increased as a result of damage to the blood/milk barrier. Similar observations were made for weakly acidic drugs like methicillin (Ziv *et al.*, 1983) and amoxycillin (Blanchflower *et al.*, 1983).

Only small differences were observed be-

tween the concentrations of carprofen in foremilk, mixed milk and aftermilk of inflamed quarters (Table II).

The same animals were used to study the pharmacokinetics of carprofen in healthy vs. mastitic cows to rule out inter-individual differences.

The elimination half-life of carprofen in healthy cows was 30.7 h, which is shorter than the half-life of 31.4 to 82.1 h of phenylbutazone (De Backer *et al.*, 1980; Martin *et al.*, 1984), but considerably longer than that of flunixin meglumine (8.1 h) (Hardee *et al.*, 1985).

This long-acting property of carprofen may be considered a therapeutic advantage over flunixin meglumine, which requires frequent dosing.

REFERENCES

Abdullah, A.S. & Baggot, J.D. (1986) Influence of induced disease states on the disposition kinetics of imidocarb in goats. *Journal of Veterinary Pharmacology and Therapeutics*, **9**, 192–197.

Anderson, K.L., Smith, A.R., Shanks, R.D., Davis, L.E. & Gustafson, B.K. (1986) Efficacy of flunixin meglumine for the treatment of endotoxin-induced bovine mastitis. *American Journal of Veterinary Research*, **47**, 1366–1372.

Ascalone, V. & Dal Bo, L. (1983) Rapid and simple determination of carprofen in the plasma by high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography*, **276**, 230–236.

Blanchflower, S.E. (1983) Antibiotic concentrations in milk from normal, endotoxin challenged, and mastitic quarters of cows after parenteral dosing with amoxycillin. *Veterinary Research Communications*, **7**, 259–260.

Blatteis, C.M., Hales, J.R.S. & Fawcett, A.A. (1988) Fever and regional blood flows in wethers and parturient ewes. *Journal of Applied Physiology*, **65**, 165–172.

De Backer, P., Breackman, R., Belpaire, F. & De Backere, M. (1980) Bioavailability and pharmacokinetics of phenylbutazone in the cow. *Journal of Veterinary Pharmacology and Therapeutics*, **3**, 29–33.

Eberhart, R.J., Natzke, R.P., Newbould, F.H.S. & Nonnecke, B. (1979) Coliform mastitis: a review. *Journal of Dairy Science*, **62**, 1–23.

Gaut, Z.N., Baruth, H., Randall, L.O., Ashley, C. & Paulsrud, J.R. (1975) Stereoisomeric relationship among anti-inflammatory activity, inhibition of platelet aggregation, and inhibition of prostaglandin synthetase. *Prostaglandins*, **10**, 59–66.

Gibaldi, M. & Perrier, D. (1982) Pharmacokinetics. 2nd edn. Marcel Dekker Inc., New York.

Hardee, G.E., Smith, J.A. & Harris, S.J. (1985) Pharmacokinetics of flunixin meglumine in the cow. *Research in Veterinary Science*, **39**, 110–112.

Hutt, A.J. & Caldwell, J. (1984) The importance of stereochemistry in the clinical pharmacokinetics of the 2-arylpropionic acid non-steroidal anti-inflammatory drug. *Clinical Pharmacokinetics*, **9**, 371–373.

International Dairy Federation (1986) Somatic cells in milk: their significance and recommended methods for counting. *Bulletin*. IDF, Brussels, Belgium.

Lees, P., Higgins, A.J., Sedgwick, A.D. & May, S.A. (1988) Applications of equine models of acute inflammation. *Veterinary Record*, **120**, 522–529.

Lohuis, J.A.C.M., Van Leeuwen, W., Verheijden, J.H.M., Brand, A. & Van Miert, A.S.J.P.A.M. (1989) Flunixin meglumine and flurbiprofen in cows with experimental *Escherichia coli* mastitis. *Veterinary Record*, **124**, 305–308.

Ludwig, B., Jordan, J.C., Rehm, W.F. & Thun, R. (1989) Carprofen in veterinary medicine. I. Plasma disposition, milk excretion and tolerance in milk-producing cows. *Schweizer Archiv für Tierheilkunde*, **131**, 99–106.

Martin, K., Anderson, L., Strindberg, M., Wiese, B. & Appelgren, L.E. (1984) Plasma concentration, mammary excretion, and side-effects of phenylbutazone after repeated oral administration in healthy cows. *Journal of Veterinary Pharmacology and Therapeutics*, **7**, 131–138.

National Mastitis Council (1981) Microbial procedures for use in the diagnosis of bovine mastitis. 2nd edn. Carter Press Inc., Ames, IA.

Peck, C.C., Beal, S.L., Sheiner, L.B. & Nichols, A.I. (1984) Extended least squares nonlinear regression: a possible solution to the 'choice of weights' problem in analysis of individual pharmacokinetic data. *Journal of Pharmacokinetics and Biopharmacology*, **12**, 545.

Randall, L.O. & Baruth, H. (1976) Analgesic and anti-inflammatory activity of 6-chloro-alpha-methyl-carbazole-2-acetic acid (C-5720). *Archives Internationales de Pharmacodynamie et de Thérapie*, **220**, 94–114.

Sheiner, L.B. & Beal, S.L. (1985) Pharmacokinetic parameter estimates from several least squares procedures: superiority of extended least squares. *Journal of Pharmacokinetics and Biopharmacology*, **13**, 18.

Strub, K.M., Aeppli, L. & Muller, R.K.M. (1982) Pharmacological properties of carprofen. *European Journal of Rheumatology and Inflammation*, **5**, 478–487.

Teelmann, K. (1983) Die Magen-Darm-Empfindlichkeit des Hundes gegenüber nichtsteroidalen Antiphlogistika/Antirheumatika. *Kleintierpraxis*, **28**, 177–188.

Van Gogh, H., Watson, A.D.J., Nouws, J.F.M., Nieuwenhuijs, J. & Van Miert, A.S.J.P.A.M. (1989) Effect of tick-borne fever (*Ehrlichia phagocytophila*) and trypanosomiasis (*Trypanosoma brucei* 1066) on the pharmacokinetics of sulfadi-

midine and its metabolites in goats. *Drug Metabolism and Disposition*, **17**, 1–6.

Verheijden, J.H.M., Schotman, A.J.H., Van Miert, A.S.J.P.A.M. & Van Duin, C.T.M. (1982) Plasma zinc and iron concentrations as measurements for evaluating the influence of endotoxin-neutralizing agents in *Escherichia coli* endotoxin induced mastitis. *American Journal of Veterinary Research*, **43**, 724–728.

Ziv, G., Soback, S. & Bor, A. (1983) Concentrations of methicillin in blood, normal milk and mastitic milk of cows after intramuscular injection of methicillin and tamethicillin. *Journal of Veterinary Pharmacology and Therapeutics*, **6**, 41–48.